IN THE ABSTRACT:

Please replace the paragraph beginning at line 5 of page 42 with the following rewritten paragraph:

-- The present invention provides methods for producing cloned avians by nuclear transfer and by combinations of nuclear transfer and ovum transfer. Particularly, methods of producing cloned avians employing two photon laser scanning microscopy are encompassed by the instant invention. The invention further contemplates methods of producing cloned transgenic avians by nuclear transfer.--

REMARKS

Claims 1-27 are pending in this application. Claims 1-27 were rejected. Claims 1, 5, 11, 14 and 19 have been amended. Claims 7-10, 12, 13 and 23 have been canceled. Claims 28-33 have been added. Entry of the amendment, reconsideration of the rejection, and allowance of all pending claims is respectfully requested.

The Amendment

The specification has been amended to correct minor errors and misspellings. No new matter has been added to the specification by amendment.

Claims 1, 5, 11, 14 and 19 have been amended to more precisely define the claimed invention. No new matter has been added to the claims by amendment. The amendments are supported by the application as filed.

Claim 1 has been amended and is now drawn to a method of producing a reconstructed avian zygote or oocyte. Support for this amendment can be found on page 10, lines 11-12 and page 14, lines 19-20. The amendment also clarifies that the recipient cell is "selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes" and that the "donor nucleus is from the same species as the recipient

cell." Support for this amendment can be found on page 4, lines 19-20; and page 14, lines 2-13 and lines 19-20.

Claim 5 has been amended to replace the term "ablation" with the term "enucleation"; thus, finding antecedent basis for this term in claim 1. Support for this amendment can be found on page 10, lines 4-6 and page 15, lines 2-3.

Claim 11 has been amended to depend upon claim 1.

Claims 14 and 19 have been amended to be consistent with amended claim 1. Claims 28-33 are new but contain no new matter. Support for claims 28 and 30, drawn to a method of producing a cloned avian can be found on page 10, lines 16-20, and page 11, lines 1-15. The claim specifies that the "reconstructed zygote is transferred into an oviduct of a recipient female of the same species as the zygote". Support for this amendment can be found on page 11, lines 9-14 and page 24, lines 7-8.

Claims 29 and 31 are new but contain no new matter. Support for these claims, drawn to a method of producing a cloned avian selected from the group consisting of chicken, duck, turkey, quail, ostrich and pheasant can be found on page 10, lines 13-15; page 18, lines 7-11; and page 25, lines 13-15.

Claims 32 and 33 are new but contain no new matter. Support for these claims to a method of preparing an enucleated recipient cell can be found throughout the specification as originally filed, including page 10, lines 3-15; page 19, lines 3-19 and page 22, lines 5-8.

The abstract has been amended to more precisely summarize the claimed invention. No new matter was added to the abstract by amendment. The amendment is supported by the application as originally filed. Support for this amendment can be found on page 9, lines 16-17; and page 10, lines 1-4, lines 13-15, and lines 16-17.

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Rejections Under 35 U.S.C. §112

Claims 1-27 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The rejection is respectfully traversed.

The Examiner asserts that the specification fails to provide sufficient teaching or guidance to show that all cloned non-human animals, or in particular avian, could be produced by nuclear transfer. More specifically, the Examiner writes that Westhusin *et al.* show the unpredictable state of the art of nuclear transfer with regard to the unpredictable factors such as species difference, donor cells and genetic modification. Furthermore, the Examiner asserts that as the specification fails to provide any guidance or teaching for the production of any cloned non-human animal or avian, one of skill would not be able to rely upon the state of the nuclear transfer art for an attempt to produce such non-human animals.

Applicants have amended the claims and introduced new claims to more precisely define the claimed invention (*supra*). The amended and new claims are supported by the application as filed (*supra*).

The Examiner's rejection of claims 1-27 due to lack of enablement appears to be two-fold. First, the Examiner appears to assert that Applicants do not provide any guidance or teaching for the production of a cloned non-human animal or avian. Applicants have amended their claims to read on avians, obviating any arguments based upon the failure of others in non-avian nuclear transfer. Further, applicants have solved one of the most significant problems confronting those practicing the art of avian nuclear transfer, visualization of the nuclear material.

Applicants specifically teach the use of nuclear transfer via two photon laser scanning microscopy (TPLSM) (pages 18-23) for nuclear transfer in avians. Furthermore, Applicants teach ovum transfer (page 23-25) as well as *ex ovo* culture (page

25, lines 12-16) for the production of cloned avians. More specifically, Applicants teach the use of two-photon visualization in nuclear transfer, wherein the egg (*i.e.*, oocyte, ovum) is removed from an animal, the genetic material of the egg is visualized and enucleated (i.*e.*, ablated), and a donor nucleus is introduced into the egg to produce a reconstructed zygote. TPLSM is used to visualize and optionally ablate the nucleus of the recipient cell (page 19, lines 1-10). TPLSM allows for the generation of images of living, optically dense structures for prolonged periods of time, while not affecting their viability. TPLSM makes all this possible because it uses biologically innocuous pulsed near infrared light which is able to penetrate much deeper into scattering specimens (page 19, lines 15-19).

It is well known in the art that the inaccessibility of the early avian egg has always posed a significant challenge. The large size and optical density of the yolk have made visualization of the avian early embryo and its structures very difficult to achieve (page 6, lines 11-20). But in order to produce an enucleated recipient cytoplast, it is essential to visualize the metaphase II plate or pronuclei. Thus, Applicants state on page 20, lines 8-14 of the specification:

> The ability to visualize the metaphase plate or pronucleus in avian egg during nuclear transfer has so far been hindered by the presence of the yolk, which makes visualization of these nuclear structures impossible. But two photon imaging with femtosecond lasers operating in the near infrared allows visualization of nuclear structures without damaging cellular constituents, despite the unfavorable optical properties of the egg yolk.

By employing TPLSM, Applicants have overcome a significant hurdle in avian nuclear transfer, *i.e.*, visualization of the early avian egg, and thus, visualization of target structures in the early avian embryo (page 20, lines 8-20 and page 21, lines 1-12).

Nuclear transfer also requires the destruction or enucleation of the pronucleus before the nuclear donor can be introduced into the oocyte (page 21, lines 13-16). Prior art methods have primarily relied on microsurgery to accomplish pronuclear removal or enucleation (page 21, lines 17-18). In a preferred embodiment, Applicants have successfully employed TPLSM for ablation of nuclear structures which is less invasive

than microsurgery and results in higher viability of the recipient cell (page 22, lines 1-4). Thus, Applicants have overcome another significant hurdle in avian nuclear transfer. On pages 19-23, Applicants describe step-by-step how to use TPLSM in avian nuclear transfer. Following ablation of the pronucleus, the nuclear donor is introduced into the germinal disk through guided injections using episcopic illumination; the reconstructed zygote can then be surgically transferred to the oviduct of a recipient hen to produce a hard shell egg (page 22, lines 8-11). Additionally, Applicants describe a method of producing a cloned animal via nuclear transfer in combination with ovum transfer (pages 23-25). Applicants provide additional guidance through illustration via detailed examples (examples 1-3, pages 31-36), wherein Applicants elaborate on the preparation of the recipient cytoplast (incubation, injection, visualization, nuclear ablation and enucleation, and isolation of the donor nucleus); preparation of the reconstructed zygote (injection); and ovum transfer into hens. One of ordinary skill in the art would easily be able to follow Applicants' teachings in order to clone avians as described in the instant invention. Applicants respectfully remind the Examiner that neither the inclusion of working examples in the specification nor the disclosure of all possible embodiments is a prerequisite for enablement (MPEP 2164.02).

Applicants submit that the nature of the invention is complex and may require some experimentation, however, the complexity of the invention does not prevent the skilled artisan from practicing what is taught. "The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue" (*In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Applicants assert that with respect to the nature of the invention, no undue experimentation would be required in order to practice the methods disclosed herein.

Second, the Examiner appears to suggest that because of the unpredictable nature of the art of nuclear transfer, one of skill would not be able to rely upon the state of the art in order to produce such non-human animals. Applicants respectfully assert that the combination of Applicants' teachings and the state of the nuclear transfer art would enable the skilled artisan to produce the cloned avians of the instant invention.

Applicants' teachings alone (*supra*) provide explicit guidance of how to clone avians, in particular chickens. Since the courts have repeatedly held that a "patent need not teach, and preferably omits, what is well known in the art" (*Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Company et al.*, 221 USPQ 481 (Fed. Cir. 1984); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986)), the specific teachings of nuclear transfer via TPLSM of every single avian species should not be a requirement for enablement.

The Examiner writes that the method steps of the claims do not enable the claimed invention, because the claims do not provide a step of transferring the reconstructed zygote into a recipient female to allow the zygote to come to term. The Examiner also writes that the recipient female must be of the same species as the zygote. Applicants have amended the claims to more precisely define the invention (*supra*) by requiring that the donor nucleus be from the same species as the recipient cell and added a step for transferring the reconstructed zygote into a recipient female of the same species as the zygote. Applicants also refer the Examiner to page 25, lines 12-16 of the specification and claim 8 which reflect that *ex ovo* culture can also be employed to bring the zygote to term.

The Examiner states that recipient cells commonly used for nuclear transfer are oocytes arrested at metaphase II and pronuclear zygotes and that it would not be predicted that use of any other enucleated recipient cell would result in successful nuclear transfer. Applicants have amended the claims to more precisely define the invention (*supra*) and clarified that the recipient cell is selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes (see amended claim 1).

The Examiner asserts that the method steps do not enable the claimed invention because they do not describe a step of cell-cell fusion. The Examiner argues that it is well known in the nuclear transfer art that cell-cell fusion must take place in order to effect nuclear transfer. Applicants refer the Examiner to page 10, lines 9-11, wherein the specification states that the donor nucleus is inserted into the recipient cell by cell fusion, microinjection, or other renucleation procedure. On page 23, lines 4-10, the specification

states that in a preferred embodiment, the cytoplasmic membrane of the cell used as nuclear donor is disrupted to expose its nucleus to the ooplasm of the recipient cytoplast. The **nuclear donor may be injected into the germinal disk**, where it undergoes reprogramming and becomes the nucleus of the reconstructed one-cell embryo. **Alternatively, a donor cell may be fused to the recipient cell using methods well known in the art**, *e.g.*, by means of fusion-promoting chemicals, such as polyethylene glycol, inactivated viruses, such as Sendai virus, or electrical stimulation. In example 3 (page 34, lines 5-17), Applicants describe the preparation of the reconstructed zygote, wherein localization and positioning of the germinal disk under the microscope and subsequent **guided injection** of the somatic cells are described (lines 13-15). Clearly, Applicants' example used injection rather then cell-cell fusion to introduce the donor nucleus into the recipient cell. Hence, Applicants' invention is not limited to cell-cell fusion.

The Examiner also asserts that the art of transgenics is unpredictable because the method of gene transfer typically relies on random integration of the transgene construct, and the expression of the transgene and the effect of transgene expression on the phenotype of the transgenic animal depends on the particular gene construct used, to an unpredictable extent. The Examiner concludes that undue experimentation is required for one skilled in the art to make and/or use the claimed invention. Applicants respectfully disagree with the Examiner's assessment that undue experimentation would be required in order to practice the claimed invention. As stated by Westhusin, et al. in the reference relied upon by the Examiner: "Work involving other species is currently ongoing, and information gathered to date suggests a wide variety of different animal species can be cloned by nuclear transplantation." (Page 36, lines 14-16). Clearly, the skilled artisan does not agree with the Examiner.

Claims 3 and 5 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The rejection is respectfully traversed.

The Examiner asserts that the term "about" in claim 3 is a relative term which renders the claim indefinite as the term "about" is not defined by the claim and the specification does not provide a standard for ascertaining the requisite degree. Applicants refer the Examiner to page 19, lines 17-21 and page 20, lines 1-3, where Applicants discuss that TPLSM utilizes biologically innocuous pulsed near infrared light, usually at a wavelength of about 700 to about 1000 nm, which is able to penetrate much deeper into scattering specimens. Applicants explain that TPLSM may employ different lasers, such as a mode-locked laser, where the wavelength is fixed, or a tunable laser that can be tuned between about 700 and about 1000 nm, depending upon the range of emission of the dye used. For DAPI and Hoescht 33342 dyes, 720-770 nm is preferred (page 20, line 1). On page 32, lines 14-19 (example 1), Applicants describe visualization of the avian early embryo through TPLSM, wherein the germinal disk was placed under the microscope objective and pronuclear structures were searched at a wavelength of 750 nm. Thus, the specification provides ample guidance for the skilled artisan to ascertain the appropriate wavelength in order to visualize nuclear material of the recipient cell using light in the near-infrared region from about 700 to about 1000 nm.

> Whether a claim is invalid for indefiniteness depends on whether those skilled in the art would understand the scope of the claim when the claim is read in the light of the specification. *Breuer Electric Mfg. Co. v. Tennant Co.*, 44 USPQ 2d 1259, 1266 (I11.1997).

Applicants assert that the language of the claims is both clear and definite and one of ordinary skill in the art would readily be able to discern the scope and meaning of the claim as written. "Breadth of a claim should not be equated with indefiniteness" (MPEP 2173.04, *In re Miller*, 169 USPQ 597 (CCPA 1971)). There is no ambiguity as to what is meant by "about 700 to about 1000 nm" since this range is clearly defined in the specification. It is common practice to use the term "about" in defining ranges.

> MPEP 2173.05(b) states the following:
> The term "about" used to define the area of the lower end of a mold as between 25 to about 45 % of the mold entrance was held to be clear, but flexible. *Ex parte Eastwood*, 163 USPQ 316 (Bd. App. 1968).

The Examiner also indicates that there is insufficient antecedent basis for the term "ablation" in claim 5. Hence, Applicants have amended the claim, replacing "ablation" with the term "enucleation" (*supra*).

Rejections Under 35 U.S.C. §102

Claim 23 was rejected under 35 U.S.C. §102(b) as being anticipated by Hughes et al. (U.S. Patent No. 4,997,763). Applicants have canceled claim 23 without prejudice.

CONCLUSION

In view of the foregoing amendment and remarks, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400, extension 5469.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at line 16 of page 2 has been amended as follows:

The ability to produce live offspring by nuclear transfer from cultured somatic cells also provides a route for the precise genetic manipulation of animal species. Such modifications include the addition or "knock-in" of genes, and the removal or inactivation or "knock-out" of genes or their control sequences (Polejaeva *et al.*, Theriogenology, 53(1):117-26, 2000). Gene targeting techniques also promise the generation of transgenic animals in which specific genes coding for endogenous proteins have been replaced by human genes coding for exogenous human proteins. In 1993, Yom and Bremmel suggested that genes coding for major proteins in cow's milk could be replaced by human counterparts. Cows modified in this fashion would produce milk containing human milk proteins, which may be more nutritious for human infants and more suitable for infant formula manufacture (Yom, H.C. and Bremmel, R.D., American Journal of Clinical Nutrition, 1993, 58 (Supplement) 306S ~~3060S~~). Methods for producing exogenous proteins in the milk of pigs, sheep, goats and cows have been reported.

The paragraph beginning at line 7 of page 28 has been amended as follows:

In one embodiment of the instant invention, a nuclear donor cell is transfected with a vector construct that contains a transgene. Methods for transfection of somatic cell nuclei are well known in the art and include, by way of example, the use of retroviral vectors, retrotransposons, adenoviruses, adeno-associated viruses, naked DNA, lipid-mediated transfection, electroporation and direct injection into the nucleus. Such techniques, particularly as applied to avians, are disclosed in Bosselman (U.S. Patent No. 5,162,215), Etches (PCT Publication No. WO99/10505), Hodgson (U.S. Patent No.

6,027,722), Hughes (U.S. Patent No. 4,997,763), Ivarie (PCT Publication No. WO99/19472), MacArthur (PCT Publication No. WO97/47739), Perry (U.S. Patent No. 5,011,780), Petitte (U.S. Patent Nos. 5,340,740 and 5,656,479 ~~5,656,749~~), and Simkiss (PCT Publication No. WO90/11355), the disclosures of which are incorporated by reference herein.

The paragraph beginning at line 1 of page 30 has been amended as follows:

In another embodiment of the instant invention, a knock-in animal has been manipulated such that it carries a specific nucleic acid sequence such as a "knock-in sequence" in a predetermined coding or noncoding region of its genome. The knock-in sequence may replace all or part of an endogenous gene of the animal by a functional homologous gene or gene segment of another animal. Knock-in animals can be prepared according to a variation of the standard knock-out method, comprising the introduction of a foreign gene into the targeting vector, in such a way that the introduced gene would be under the control of the regulatory elements that normally control the expression of the endogenous gene (~~Le,~~ Le Mouellic *et al.*, *Proc. Natl. Acad. Sci. USA* 87:4712-6, 1990) and (McCreath *et al.*, *Nature* 405:1066-1069, 2000).

The paragraph beginning at line 16 of page 31 has been amended as follows:

Ova were isolated from euthanized hens between 2-4 hours after oviposition of the previous egg. Alternatively, eggs were isolated from hens whose oviducts have been fistulated (Gilbert and Woodgush, *Journal of Reproduction and Fertility* 5:451-453, 1963) and (~~Pander, et al.~~ Pancer *et al.* *Br. Poult. Sci.* 30:953-7, 1989).

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In the Claims:

Claim 1 is amended as follows:

1. (Amended) A method of producing a ~~cloned non-human animal~~ reconstructed avian zygote or oocyte comprising the steps of:

(i) providing a recipient cell selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes;

(ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;

(iii) enucleating the recipient cell; and

(iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce ~~a~~ the reconstructed avian zygote or oocyte.

~~(v) activating the reconstructed zygote; and~~

~~(vi) allowing the reconstructed zygote to develop to term.~~

Claim 5 is amended as follows:

5. (Amended) The method of claim 1, wherein the visualization and ~~ablation~~ enucleation are conducted using two photon laser scanning microscopy.

Claims 7-10 are canceled.

Claim 11 is amended as follows:

11. (Amended) The method of claim [10] 1 wherein the [cloned] avian is selected from the group consisting of chickens, ducks, turkeys, quails, ostriches and pheasants.

Claims 12 and 13 are canceled.

Claim 14 is amended as follows:

14. (Amended) A method of producing a cloned ~~non-human animal~~ avian comprising the steps of:

(i) providing a recipient cell selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes;

(ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;

(iii) enucleating the recipient cell using light in the near infrared region;

(iv) introducing a donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed avian zygote or oocyte.

(v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;

(vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and

(vii) allowing the reconstructed zygote or oocyte to develop to term.

Claim 19 is amended as follows:

19. (Amended) A method of producing a transgenic avian comprising the steps of:

(i) providing an avian recipient cell selected from the group consisting of avian oocytes arrested at metaphase II and pronuclear zygotes;

(ii) visualizing the nuclear material of the recipient cell using light in the near-infrared region;

(iii) enucleating the recipient cell;

(iv) introducing a transgenic avian donor nucleus from the same species as the recipient cell into the recipient cell to produce a reconstructed avian zygote or oocyte.

(v) activating the reconstructed zygote or fertilizing the reconstructed oocyte;

(vi) transferring the reconstructed zygote or fertilized oocyte into an oviduct of a recipient female of the same species as the zygote or oocyte; and

(vii) allowing the reconstructed zygote or oocyte to develop to term.

Claim 21 is amended as follows:

21.(Amended). A method of producing a protein, comprising:

(i) producing a transgenic avian according to the method of claim [20] 19 wherein the transgene encodes an exogenous protein, said protein deposited in the white of the developing eggs of said avian;

(ii) harvesting hard shell eggs; and

(iii) isolating the exogenous protein from said eggs.

Claim 23 is canceled.

New claims 28-33 have been added:

28. (New) A method of producing a cloned avian comprising:

(i) producing a reconstructed zygote by the process of claim 1;

(ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and

(iii) allowing the reconstructed zygote to develop to term.

29. (New) The method of claim 28, wherein the cloned avian is selected from the group consisting of chicken, duck, turkey, quail, ostrich and pheasant.

30. (New) A method of producing a cloned avian comprising:

(i) producing a reconstructed oocyte by the process of claim 1;

(ii) fertilizing the reconstructed oocyte to produce a reconstructed zygote;

(ii) transferring the reconstructed zygote into an oviduct of a recipient female of the same species as the zygote; and

(iii) allowing the reconstructed zygote to develop to term.

31. (New) The method of claim 30, wherein the cloned avian is selected from the group consisting of chicken, duck, turkey, quail, ostrich and pheasant.

32. (New) A method of preparing a recipient cell comprising the steps of:

(i) providing a cell having a nucleus therein;

(ii) visualizing the nucleus using light in the near-infrared region; and

(iii) ablating the nucleus to provide an enucleated recipient cell.

33. (New) The method of claim 32 wherein the nucleus is visualized and ablated via two photon laser scanning microscopy.

In the Abstract:

The paragraph beginning at line 5 of page 42 has been amended as follows:

The present invention provides methods ~~Methods~~ for producing cloned [non-human animals] avians by nuclear transfer and by combinations of nuclear transfer and ovum transfer. ~~,employing near-infrared visualization of the recipient cell nucleus are described herein. Transgenic, knock-out, and knock-in avians are provided. Methods for producing eggs which contain exogenous proteins are encompassed by the present invention. Thus, the instant invention satisfies the need for an effective route to the generation of cloned avians~~. Particularly, methods of producing cloned avians employing two photon laser scanning microscopy are encompassed by the instant invention. The invention further contemplates methods of producing cloned transgenic avians [produced by the methods described herein] by nuclear transfer.